

REPORT DOCUMENTATION PAGE

Form Approved
OMB NO. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comment regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 2 March 1998		3. REPORT TYPE AND DATES COVERED Final Report, 14 June 1990 to Sept. 1997	
4. TITLE AND SUBTITLE Mechanisms of Resistance in Microbial Spores				5. FUNDING NUMBERS DAAL03-90-G-0146	
6. AUTHOR(S) Philipp Gerhardt and Robert E. Marquis (Co-Principal Investigators)					
7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES) Department of Microbiology Michigan State University East Lansing, MI 48824				8. PERFORMING ORGANIZATION REPORT NUMBER 11 (Final)	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211				10. SPONSORING / MONITORING AGENCY REPORT NUMBER ARO 28022.16-LS	
11. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.					
13. ABSTRACT (Maximum 200 words) Pursuant to the goals and objectives of the project, the following principal results were obtained: (1) the native peptidoglycan in the cortex around the dormant spore protoplast has low electrical conductivity and is highly cross-linked, contrary to results usually reported; (2) the water within the entire spore is in a free state and exchangeable by diffusion with external water; (3) hydrostatic pressure enhances the heat killing of spores; (4) the primary targets in the thermal inactivation of spores are crucial proteins of the protoplast; (5) the sporocidal mechanism of action of heat and peroxides is oxidation involving the formation of chemical radicals; (6) mineralization of spores increases their resistance to thermal inactivation; (7) polyhydroxybutyrate occurs in spores only dispersed, complexed with other biopolymers, in small amounts, and at low molecular mass; (8) a major book on basic methods for general and molecular bacteriology was edited and authored in part by the co-principal investigators; (9) claims for the revival of bacterial spores and even vegetative cells after desiccation for millions of years within amber were assessed.					
14. SUBJECT TERMS BACTERIAL SPORES, RESISTANCE MECHANISMS				15. NUMBER OF PAGES 4	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OR REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL		

MECHANISMS OF RESISTANCE IN MICROBIAL SPORES
Final Report, 2 March 1998

Philipp Gerhardt
Department of Microbiology
Michigan State University
East Lansing, MI 48824

Robert E. Marquis
Department of Microbiology
University of Rochester
Rochester, NY 14642

U.S. Army Research Office
Grant Number DAAL03-90-G-0146
14 June 1990 to 13 September 1997

Approved for Public Release
Distribution Unlimited

1. GOALS AND OBJECTIVES

The long-range goals of the project were to identify and explain the physicochemical determinants and physiological mechanisms accounting for heat resistance and the targets and mechanisms accounting for thermal inactivation of dormant bacterial spores.

The specific objectives for the 8¼-year duration of the project were to investigate quantitatively the following problems on spore heat resistance:

1. Molecular characterization of the cortical peptidoglycan of dormant bacterial spores;
2. Water states within spore structures;
3. Hydrostatic pressure as an adjuvant in heat killing of spores;
4. Sequence of structural and molecular targets of irreversible thermal inactivation of spores;
5. Oxidation as a molecular mechanism in heat and peroxide killing of spores;
6. Mineralization of spores as a factor in thermal inactivation;
7. Re-assessment of the occurrence of polyhydroxybutyrate in spores;
8. Preparation of a book on basic methods for general and molecular bacteriology, including spores;
9. Assessment of claims for the revival of bacterial spores after desiccation for millions of years within amber.

2. SUMMARY/ABSTRACT OF RESULTS

Pursuant to the goals and objectives of the project, the following principal results were obtained: (1) the native peptidoglycan in the cortex around the dormant spore protoplast has low electrical conductivity and is highly cross-linked, contrary to results usually reported; (2) the water within the entire spore is in a free state and exchangeable by diffusion with external water; (3) hydrostatic pressure enhances the heat killing of spores; (4) the primary targets in the thermal inactivation of spores are crucial proteins of the protoplast; (5) the sporocidal mechanism of action of heat and peroxides is oxidation involving the formation of chemical radicals; (6) mineralization of spores increases their resistance to thermal inactivation; (7) polyhydroxybutyrate occurs in spores only dispersed, complexed with other biopolymers, in small amounts, and at low molecular mass; (8) a major book on basic methods for general and molecular bacteriology was edited and authored in part by the co-principal investigators; (9) claims for the revival of bacterial spores and even vegetative cells after desiccation for millions of years within amber were assessed.

3. LIST OF PRIMARY PUBLICATIONS

3.1. FROM UNIVERSITY OF ROCHESTER

Marquis, R. E. 1993. Bacteria, p. 1-28. *In* Macdonald, A. G. (ed.), Adv. Comp. Environ. Physiol., Vol. 17, Effects of high pressure on biological systems. Springer-Verlag, Berlin.

Marquis, R. E. 1994. High pressure microbiology, p. 1-14. *In* Bennett, P. B., and R. E. Marquis (ed.). Basic and applied high pressure biology. Univ. of Rochester Press, Rochester, NY.

Shin, S. Y., E. G. Calvisi, T. C. Beaman, P. Gerhardt, and R. E. Marquis. 1994. Microscopic and thermal characterization of hydrogen peroxide killing and lysis of spores and protection by transition metal ions, chelators, and antioxidants. *Appl. Environ. Microbiol.* 60:3192-3197.

Marquis, R. E., and S. Y. Shin. 1994. Mineralization and responses of bacterial spores to heat and oxidative agents. *FEMS Microbiol. Rev.* 14:375-380.

Shin, S. Y., and R. E. Marquis. 1994. Sporicidal activity of tertiary butyl hydroperoxide. *Arch. Microbiol.* 161:184-190.

Marquis, R. E., J. Sim, and S. Y. Shin. 1994. Molecular mechanisms of resistance to heat and oxidative damage. *J. Appl. Bacteriol. Symp. Suppl.* 76:40S-48S.

Marquis, R. E. 1994. Permeability and transport, p. 587-599. *In* P. Gerhardt *et al.* (ed.). Methods for general and molecular bacteriology. Amer. Soc. Microbiol., Washington, DC.

Marquis, R. E., G. C. Rutherford, M. M. Faraci, and S. Y. Shin. 1995. Sporicidal action of peracetic acid and protective effects of transition metal ions. *J. Indust. Microbiol.* 15:486-492.

3.2. FROM MICHIGAN STATE UNIVERSITY

Belliveau, B. H., T. C. Beaman, H. S. Pankratz, and P. Gerhardt. 1992. Heat killing of bacterial spores analyzed by differential scanning calorimetry. *J. Bacteriol.* 174:4463-4474.

Gerhardt, P., R.G.E. Murray, W. A. Wood, and N. R. Krieg (ed.). 1994. Methods for general and molecular bacteriology, 791 pp. Amer. Soc. Microbiol., Washington, DC.

Gerhardt, P. 1994. Methodology for general and molecular bacteriology, p. 1-2. *In* P. Gerhardt *et al.*, *ibid.*

Gerhardt, P. 1994. Introduction to growth, p. 135. *In* P. Gerhardt *et al.*, *ibid.*

Krieg, N. R., and P. Gerhardt. 1994. Solid, liquid/solid, and semisolid culture, p. 216-223. *In* P. Gerhardt *et al.*, *ibid.*

Gerhardt, P., and S. W. Drew. 1994. Liquid culture, p. 224-247. *In P. Gerhardt et al., ibid.*

Gerhardt, P. 1994. Diluents, p. 255. *In P. Gerhardt et al., ibid.*

Gerhardt, P. 1994. Biomass measurement, p. 260-261. *In P. Gerhardt et al., ibid.*

Gerhardt, P. 1994. Introduction to molecular genetics, p. 295-296. *In P. Gerhardt et al., ibid.*

Gerhardt, P. 1994. Introduction to general methods, p. 713. *In P. Gerhardt et al., ibid.*

Gerhardt, P. 1994. Records and reports, p. 763-766. *In P. Gerhardt et al., ibid.*

Reusch, R. N., R. Huang, and P. Gerhardt. 1997. Only complexed polyhydroxybutyrate is in bacterial spores. *Microbiol.* 143:2851-2852.

Gerhardt, P. 1998. Survival of ancient bacteria desiccated within amber: believe it or not? Controversy persists whether bacteria trapped within amber really are preserved and revivable after millions of years. *ASM News* 64:68-69.

4. LIST OF PERSONNEL

4.1. AT UNIVERSITY OF ROCHESTER

Robert E. Marquis
Soon Young Shin (accomplished postdoctoral training)
Jimmy Sim (earned B.S. degree)
Glen C. Rutherford (pursuing M.S. degree)
Michelle Faraci (earned B.A. degree)
Alfredo Palop (accomplished postdoctoral training)

4.2. AT MICHIGAN STATE UNIVERSITY

Philipp Gerhardt
Brian Belliveau (accomplished postdoctoral training)
Teofila C. Beaman
H. Stuart Pankratz
Rosetta N. Reusch
Ruiping Huang (accomplished postdoctoral training)

5. INVENTIONS

No inventions from this project were applied for or received.